Attenuated Live Bluetongue Virus 8 Vaccine Protects Sheep from Challenge with the European BTV-8

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Abstract: An attenuated live bluetongue virus (AL-BTV) serotype 8 vaccine of South African origin was evaluated for its ability to protect sheep against challenge with a European BTV-8 isolated from an outbreak. Two groups of sero-negative sheep were vaccinated with either a single or booster vaccination and challenged subcutaneously 28 days following vaccination.

Groups of vaccinated challenged sheep showed no clinical signs typical to Bluetongue disease, as compared to unvaccinated inoculated sheep of a control group. A clinical reaction index (CRI) of values ranging from 0 to 5 was determined for each of the groups, where vaccinates measured a CRI of less than 1 and the control sheep measured a CRI of at least 4.5.

All vaccinated sheep developed high serotype-specific neutralizing antibodies post-vaccination. The results indicated that a single dose of the AL-BTV-8 was sufficient to protect vaccinated sheep against a virulent challenge of the European BTV-8 outbreak isolate.

INTRODUCTION

The control of Bluetongue disease, an insect-borne multi-serotype viral disease of sheep, cattle, goat and other ruminants, relies essentially on vaccination. In South Africa, where 21 of the 24 known serotypes have been isolated [1] a multivalent attenuated live Bluetongue virus (AL-BTV) vaccine consisting of a mix of multiple BTV serotype has been used over the years to protect exotic sheep breeds which are highly susceptible to the disease. Taking advantage of the cross-neutralization and subsequent cross-protection between certain BTV serotypes [2], the currently used multivalent vaccine consists of 15 serotypes, grouped into 3 pentavalent vaccines, administered as a course of 3 separate injections given intervals of 3 weeks [2].

Since the recent introduction of BTV in Europe, and more specifically the spread of BTV-8 to several European countries, vaccination has been advocated as one of the key control measures. Affected countries are using the inactivated BTV-8. Although live attenuated viral vaccines are known to trigger a long lasting immunity following a single vaccination, the attenuated live BTV-8 vaccine (AL-BTV-8) is not being used in Europe mainly due to safety concerns. In order to confirm the ability of the AL-BTV-8 vaccine strain to protect sheep against a virulent BTV-8 involved in the North European BT outbreak [3], a monovalent AL-BTV-8 vaccine was produced and used to vaccinate susceptible sheep.

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MATERIALS AND METHODS

Ten BTV-naïve Merino sheep aged 1-2 years were held in isolation in insect-free stables for the duration of the trial. They were divided into two groups of four sheep and vaccinated with the monovalent AL-BTV-8 vaccine; two sheep served as unvaccinated controls. The monovalent AL-BTV-8 vaccine was duly produced and tested at OBP Ltd. Sheep in Groups 1 and 2 were vaccinated subcutaneously with 1ml of reconstituted freeze-dried vaccine with a minimum dose of 10^4 pfu/ml. Sheep in Group 2 received a booster vaccination on day 28.

A low cell culture passaged European sheep isolate designated as BTV-8 (G) 5/10A 2006/01KC 3BHK, isolated from a BTV outbreak in the Netherlands, was obtained through the OIE Reference Laboratory for BTV, ARC-Onderstepoort Veterinary Institute, and used to challenge vaccinated sheep. Virulent virus suspension of an additional passage in BHK21-C13 cells with a dose of 10^7 pfu/ml was administered intravenously. Group 1 was challenged on day 28 post-vaccination and Group 2 on day 28 post-booster vaccination. In order to mimic natural conditions and exacerbate the disease sheep were exposed to the environment and sunlight for 3-4 hours daily post challenge [4]. Following restrictions imposed by the Animal Ethics committee, only two unvaccinated sheep were inoculated with virulent virus and served as control in measuring clinical symptoms resulting from BTV infection.

Vaccine safety was demonstrated by monitoring febrile reactions and observing sheep for clinical signs typical to BTV for 14 days post-vaccination and 14 days post-challenge. The clinical reaction index (CRI) developed for the evaluation of BTV vaccine was used to quantify the observed clinical reactions post-challenge of vaccinated and

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post-inoculation of control sheep [5, 6]. In short the CRI score (0-5) was calculated as the sum of the following: temperature score, i.e. the cumulative total of fever readings above 40°C on days 3 to 14 post-challenge; clinical lesion score, i.e. lesions of the mouth, nose and hooves each scored on a scale of 0-4; death, i.e. an additional 4 points where death occurred within 14 days post challenge.

Sheep were bled post-vaccination and post-challenge every 3 to 4 days for 21 days collecting blood in heparin-coated vacu-tubes and blood was collected for serum on a 7 day interval. Viraemia was determined by virus isolation on Vero cell culture and neutralizing antibody titers to BTV-8 were measured using the serum neutralization test (SNT) [7].

RESULTS

Sheep vaccinated with the AL-BTV-8 vaccine showed no rise in their normal body temperatures of 39.5-40°C. Only one sheep in Group 1 showed a febrile reaction of >40°C on days 3 and 6 (sheep #10), whereas control sheep #C145 and #C151 showed febrile reactions of >40°C from day 4 to day 7 after inoculation with virulent BTV-8 (Fig. **1**).

No clinical signs were observed in vaccinated sheep following challenge. Control sheep however developed slight hyperemia of the nasal and buccal mucosa and slight erosion in the mouth, noticeable between days 5 and 9 post-challenge. As shown in Fig. (**2**), the CRI of vaccinated-challenged sheep in Group 1 were all 0, except for sheep #10, which had a CRI of 0.1 due to the febrile reaction. Control sheep #C145 and #C151 had a CRI of 5 and 4.5 respectively.

Replicating BTV-8 could not be isolated by repeated blind passaging in cell culture from heparinised blood collected from sheep post-vaccination and post-challenge. Viraemia was detected in control sheep on days 5 to 7 following inoculation with virulent virus. Sero-conversion to BTV-8 was evident soon after one vaccination reaching a peak by day 21. Sheep in Group 1 that received a single dose of AL-BTV-8 were well protected with neutralizing antibody titers (nAb) of at least 1:256 on the day of challenge (day 28). nAb titers doubled by day 14 post-challenge with titers as high as 1:1024 (day 49). Serum neutralizing titer results are presented in Table **1**. Sheep in Group 2 sero-converted following the first vaccination and booster vaccination with AL-BTV-8. nAb titers gradually increased over a period of 28 days reaching titers of 1:1024. Titers remained at that level following challenge for the remainder of the test period. BTV-8 specific antibodies were detected in control sheep after inoculation (Table **1**).

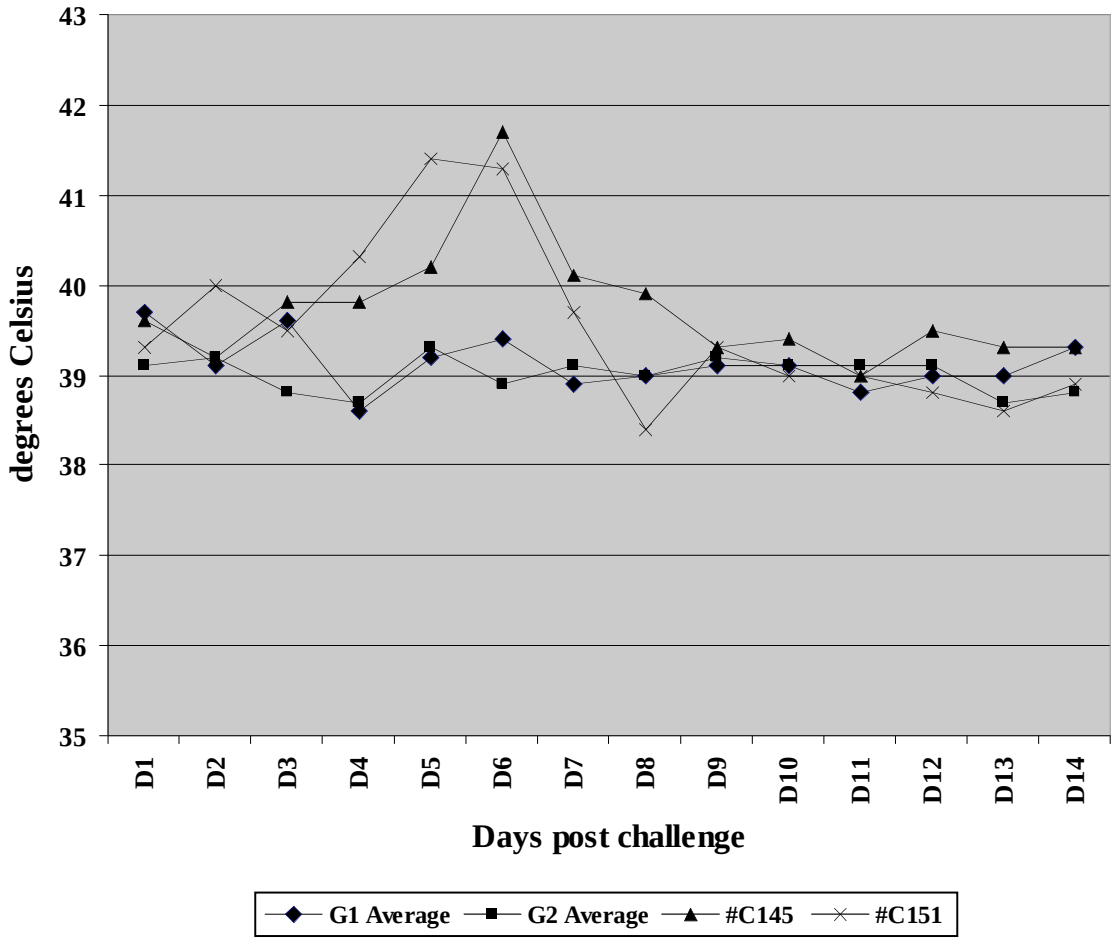

Fig. (1). Average rectal temperature recordings post-challenge with a virulent European BTV-8 isolate of sheep vaccinated with a single (Group 1) and a booster (Group 2) vaccine of AL-BTV-8. #C145 and #C151 served as control sheep.

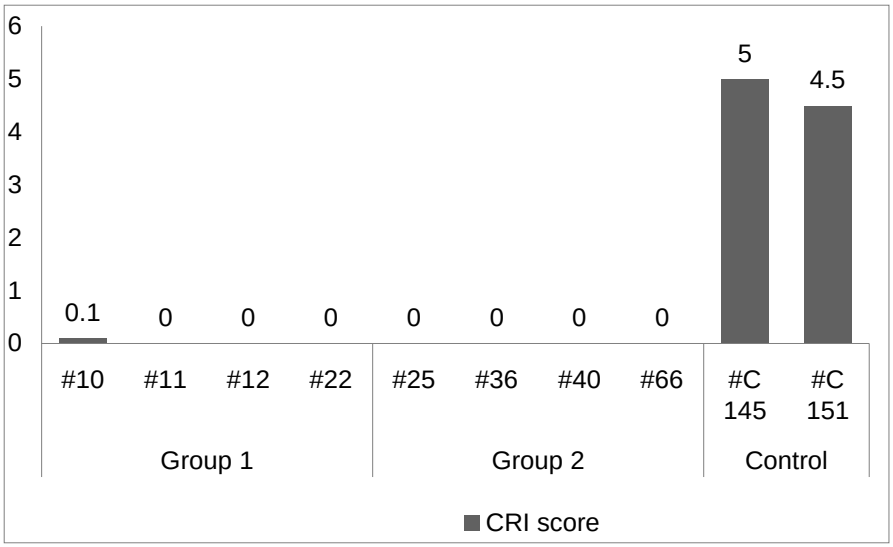

Fig. (2). Clinical Reaction Index (CRI) score post-challenge of sheep in Groups 1 and 2 vaccinated with AL-BTV-8 vaccine. Sheep #C145 and #C151 are unvaccinated controls challenged with a virulent European BTV-8 isolate.

There was no difference between the CRI of Group 1 and 2, indicating that the booster dose was not required to generate a protective immunity.

DISCUSSION

Although African sheep breeds are known to be less susceptible to BTV as compared to European breeds, the use of the CRI and other factors such as sunlight made it possible to compare the impact of the BTV infection in vaccinated and unvaccinated local Merino sheep.

The ability of the monovalent Onderstepoort BTV-8 vaccine to protect sheep against the European BTV-8 should not be unexpected. It has been established that several strains of the same serotype may circulate within the same endemic environment, and that the European BTV-8 might be of African origin [8]. The live BTV vaccine used in South Africa provides protection to different strains of the serotypes currently circulating in the country. Furthermore, monovalent and bivalent BTV vaccines based on the Onderstepoort BTV vaccine have been generated for use in Mediterranean countries affected with Bluetongue viruses phylogenetically different to the South African isolates and have resulted in protection of both sheep and cattle [9, 10].

Table 1. BTV-8 Neutralizing Antibody Titers for Sheep Vaccinated with AL-BTV-8 in Group 1 (Single Vaccination) and Group 2 (Booster Vaccination) and Sero-Conversion Following Challenge with Virulent BTV-8 (European Isolate)

	Sheep No.	Inoculation								
		D0	D21	D28						
Control	#C145	0	256	512						
#C151	0	128	256							
		Vaccination			Challenge					
		D0	D14	D21	D28	D49	D56			
Group 1	#10	0	64	512	256	256	512			
	#11	0	128	512	256	1024	1024			
	#12	0	64	512	256	1024	512			
	#22	0	128	256	512	2048	1024			
		Vaccination			Booster			Challenge		
		D0	D14	D21	D28	D42	D49	D56	D77	D84
Group 2	#25	0	64	256	512	512	512	1024	512	1024
	#36	0	32	512	128	256	512	1024	1024	1024
	#40	0	8	32	128	512	512	1024	1024	1024
	#65	0	64	512	256	512	512	2048	1024	2048

Although a small number of sheep was used in the present study, the overall vaccination-challenge results indicate that the AL-BTV-8 vaccine protected vaccinated sheep against a virulent challenge of the European BTV-8, with a single dose, while the unvaccinated control sheep showed clinical signs of the disease. The fact that a booster dose did not make a difference to the protection already achieved by a single dose vaccination is in line with the normal vaccination schedule of other AL-BTV vaccines, which are administered as a single dose [2]. The protection afforded is further confirmed by the high level of neutralizing antibody titers recorded in all vaccinated animals.

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